HYPOLIPIDAEMIC EFFECTS OF NARINGENIN, RUTIN, NICOTINIC ACID AND THEIR ASSOCIATIONS

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Atherosclerosis can be defined as being a disease of coronary circulation. The present work evaluates the action of the naringenin, rutin, nicotinic acid, isolated and in association, on the metabolism of lipids. Cholesterol, cholesterol HDL, and triacylglycerols have been dosed after retreat of blood, following the administration of the compounds dissolved in propylene glycol by intraperitoneal route in doses of 5 mg kg^{-1} body wt. Results evidence that naringenin and nicotinic acid, isolated as well as their association with naringenin and nicotinic acid−rutin, present the largest percentual reduction of cholesterol. On the other hand, the best results for cholesterol-HDL have been obtained with naringenin, while rutin has shown the best triacylglycerols levels.   © 1999 Academic Press

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INTRODUCTION

Hyperlipidaemies are traditionally defined as conditions in which lipoprotein concentrations exceed an arbitrary normal limit.

The pathogenesis of coronary and cerebrovascular thrombosis has been interpreted as a misdirected form of haemostasis in which platelets interact with subendothelium, leading to adhesion, activation, aggregation, and recruitment, and accompanied by fibrin formation. This concept evolved mainly from studies of the response of healthy blood vessels to physical injury and formed the basis for current platelet-directed therapeutic approaches to arterial thrombosis. However, thrombotic events almost always occur at sites of pathologic vascular damages, containing necrotic or lipid-rich tissue and inflammatory cells [1−3].

The current antithrombotic medications include platelet aggregation inhibitors, blood coagulation inhibitors and fibrinolitics [4].

The reduction of cholesterol in the prevention of coronary arterial disease decreases the incidence of heart attacks. Medications such as nicotinic acid have been used in the treatment of hypercholesterolaemic patients at present. These medicines work as kidnapped by bile acids, and inhibit the secretion of lipoproteins by the liver so that low density lipoproteins are reduced, including the rich component rich in triacylglycerols. The basic effect of the nicotinic acid can be a smaller mobilization of fatty free acid starting from the adiposis tissue, so that there is less substratum for hepatic synthesis of lipoproteic lipids [5].

Several researchers, among them Yugarani [6] have demonstrated that flavonoids act as reducers of lipid activities in animals, and using triton as a hyperlipidaemia inductor, Jahromi and Ray [7] determined serum lipid levels in rats after the administration of marsupin, pterosupin, and liquirritigenin. Results showed that liquirritigenin and pterosupin reduced cholesterol, cholesterol-LDL levels, and atherogenic indexes. Pterosupin also was efficient in the reduction of triacylglycerols.

Andreeva [8] verified the hypolipidemic activities of the glucosylated flavonoids extracted from *Viscia tranculata* in rats. The compounds isolated from these plants were derivative sugars of diosmetin derivatives.

Syrov [9] tested luteolin, quercetin, cynarosyl, haplogenin-7-*O*-glucosyl derivative, haplosídeo A, pec-

Table I
Average levels of cholesterol ($\pm$ SE) in the serum of Wistar rats and their respective percentual variation

Groups	Cholesterol (mg dl^{-1})	% of variation
1—Ration	26.36 ± 0.70	–
2—Ration + triton	262.11 ± 13.96b	–
3—Ration + triton + naringenin	47.57 ± 1.90	−81.85*
4—Ration + triton + rutin	103.09 ± 2.52a	−60.67*
5—Ration + triton + nicotinic acid	41.81 ± 1.00b	−84.05*
6—Ration + triton + nicotinic acid + naringenin	54.53 ± 1.07b	−79.20*
7—Ration + triton + nicotinic acid + rutin	39.68 ± 0.98b	−84.86*

*Statistic data are different differenciated from the control (ration + triton) by using Dunnett's test ($P < 0.05$).
Note: Averages followed by same letters do not differ from each other in Tukey's test ($P > 0.05$).

tolinaringenin and hispidulin in doses of 10 mg kg^{-1}. Results evidenced pronounced reduction of cholesterol, triacyilglycerols and β-lipoproteins and antiatheromatic effects.

The present work aims to evaluate the effects of naringenin, rutin, nicotinic acid, isolated and associated with flavonoids, in the control of lipidic metabolism.

MATERIAL AND METHODS

In this work male rats of the Wistar race, provided by the Department of Nutrition of Universidade Federal de Viçosa, weighing approximately 200 ± 20 g, received commercial ration, labin, and water *ad libitum*. After an adaptation period, the animals were separated in seven experimental groups with eight animals in each. They were randomly distributed to receive the following treatments:

Group 1 (ration); Group 2 (ration + triton); Group 3 (ration + triton + naringenin); Group 4 (ration + triton + rutin); Group 5 (ration + triton + nicotinic acid); Group 6 (ration + triton + naringenin + nicotinic acid); Group 7 (ration + triton + rutin + nicotinic acid).

Experimental delineation was entirely randomized for the seven treatments, in eight repetitions.

Before beginning treatments, the animals were submitted to a period of adaptation to cages with control of light and darkness of 12 h for 5 days.

In order to induce hyperlipidaemia, triton (Sigma St Louis, MO, USA), dissolved in a physiologic solution of NaCl at 0.9% was administered by the intraperitoneal route in doses of 300 mg kg^{-1} body wt. After 24 h, the flavonoids, naringenin, rutin, and the medicine, nicotinic acid from Sigma, were administered isolated and in association with the flavonoids. These compounds were supplied by the intraperitoneal route, dissolved in propylenglycol as a vehicle, in the dose of 5 mg kg^{-1} body wt. After 24 h, the animals were anaesthetized with ethyl ether by inhalation and, blood samples were taken by heart puncture. These materials were centrifuged at 7161.6 *g* min^{-1}, to obtain the serum, which was analysed for cholesterol and triacylglycerols using enzymatic kits, following Henry's method [10]. Lima's method [11] was used for cholesterol-HDL. Quantitative analysis was made with a Hitachi spectrophotometer.

RESULTS AND DISCUSSION

Results of serum lipids in rats are given in Tables I−III. They are expressed in mg dl^{-1}, with their respective percentual variations.

Table II
Average levels of cholesterol-HDL ($\pm$ SE) in the serum of Wistar rats and their respective percentual variation

Groups	Cholesterol—HDL (mg dl^{-1})	% of variation
1—Ration	22.25 ± 0.79	–
2—Ration + triton	64.20 ± 0.18	–
3—Ration + triton + naringenin	46.61 ± 2.06a	−27.40*
4—Ration + triton + rutin	40.62 ± 0.64b	−36.73*
5—Ration + triton + nicotinic acid	30.33 ± 0.50c	−52.76*
6—Ration + triton + nicotinic acid + naringenin	22.31 ± 1.38d	−65.25*
7—Ration + triton + nicotinic acid + rutin	39.96 ± 1.31b	−37.76*

*Statistic data are differenciated from the control (ration + triton) by using Dunnett's test ($P < 0.05$).
Note: Averages followed by the same letters do not differ from each other in the Tukey's test ($P > 0.05$).

Table III
Average levels of triacylglycerols ($\pm$ SE) in the serum of Wistar rats and their respective percentual variation

Groups	Triacylglycerols (mg dl^{-1})	% of variation
1—Ration	160.68 $\pm$ 2.68	–
2—Ration + triton	308.90 $\pm$ 2.82	–
3—Ration + triton + naringenin	105.06 $\pm$ 1.03b	$-65.99*$
4—Ration + triton + rutin	72.41 $\pm$ 1.43c	$-76.56*$
5—Ration + triton + nicotinic acid	102.75 $\pm$ 0.54b	$-66.74*$
6—Ration + triton + nicotinic acid + naringenin	101.13 $\pm$ 1.56b	$-67.26*$
7—Ration + triton + nicotinic acid + rutin	119.92 $\pm$ 1.19a	$-61.18*$

*Statistic data are differenciated from the control (ration + triton) by using Dunnett's test ($P < 0.05$).
Averages followed by same letters do not differ from each other in Tukey's test ($P > 0.05$).

In agreement with the results shown in Table I, the animals that received triton had cholesterol levels that increased from 26.36 ± 0.70 to 262.11 ± 13.96 mg dl^{-1}. These hypercolesterolaemics animals had cholesterol levels reduced in all treatments. The most efficient treatment was accomplished with naringenin (Group 3), nicotinic acid, isolated (Group 5) as well as in association with naringenin (Group 6), and with rutin (Group 7), which presented the largest percentual reduction, as shown by Dunnet's test.

Results of cholesterol-HDL, shown in Table II, evidence that the best treatment was accomplished with naringenin (Group 3). That is an advantage, since cholesterol-HDL is responsible for the transportation of cholesterol from peripheric tissues to the liver for metabolization.

Finally, the results in Table III for triacylglycerol analysis of serum of the rats indicate a significant reduction of its levels, with the largest percentual reduction for rutin (Group 4).

The mechanism of action seems to be related to that obtained by Sepulveda and Robinson [12]. They, verified that catechin stimulates the uptake of sugars and amino acids, and inhibits the accumulation of *p*-amino-hippurate and *N*-methyl-nicotinamide in dog renal cortex slices, by a specific effect on the permeability of the basolateral plasma membrane of tubular cells. By using naringenin, Robinson [13] verified that it inhibits the transport of sugars and amino acids in small intestine of dogs, guinea-pigs and rats. This inhibition is in part explained by an effect of naringenin on the metabolism and in part by the direct action of naringenin on cell membranes. These studies show that naringenin stimulates the Na$^+$-dependent alanine uptake in isolated enterocytes by a specific effect on the basolateral plasma membrane, which inhibits the efflux of amino acids across this membrane. The inhibitory effect is probably due to some metabolic alteration and the inhibition of Na$^+$–K$^+$ ATPase [14]. Compounds which inhibit blood platelet function are of potential use in the preventive treatment of thrombosis or atherosclerosis [15]. It is possible to imply cAMP in their mechanism of action because it inhibits cyclic nucleotide phosphodiesterase (PDE) in human platelets [16]. The metabolism of lipoproteins seems to be affected by nicotinic acid as a result of the reduction of the production of VLDL due to a transient inhibitory effect of the nicotinic acid on the lipolise, the reduced release of free fat acids to the liver and the reduction in the synthesis of triglyceride and in the VLDL–triglyceride transportation. An increased activity of lipase lipoprotein can lead to an increased VLDL depuration.

The decrease of LDL levels may occur due to the reduction of VLDL and the increase of hepatic depuration of LDL precursors [17].

It can be concluded that naringenin seems to be possibly useful in the treatment of hyperlipidaemic diseases.

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